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4-*N*-ACYLFORTIMICINS B AND THE PREPARATION OF FORTIMICIN A FROM FORTIMICIN B

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ABSTRACT

Selective 4-*N*-acylation of fortimicin B (**2**) has been accomplished by 4-*N*-acylation of 1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B (**4**) followed by hydrogenolysis of the *N*-protecting benzyloxycarbonyl groups. In this manner, fortimicin B was converted into fortimicin A (**1**), and a series of 4-*N*-acylfortimicins B (**3**) was prepared for antibacterial assay. The key intermediate, 1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B, was prepared either directly from fortimicin B or by converting fortimicin A into 1,2′,6′,2″-tetra-*N*-benzyloxycarbonylfortimicin A (**6a**), followed by selective hydrolysis of the 4-*N*-(*N*-benzyloxycarbonyl)glycyl group of the latter.

INTRODUCTION

The aminocyclitol antibiotics fortimicin A (**1**) and fortimicin B (**2**) are formed in fermentations by *Micromonospora olivoasterospora*[1]. Structural studies[2] have established that these compounds are pseudodisaccharides composed of a diaminosugar (6′-*epi*-purpurosamine B) attached by a glycosidic bond to O-6 of a 1,4-diaminocyclitol (fortamine). Structurally, fortimicin A differs from fortimicin B in that the former has a glycyl group attached by an amide bond to the 4-methylamino group of the cyclitol. Detailed studies of the n.m.r. spectra[2] of the fortimicin free bases have established that, in aqueous solution, fortimicin A adopts that chair conformation in which the 4-$NCH_3COCH_2NH_2$ group is equatorial, whereas fortimicin B adopts that chair conformation in which the 4-$NHCH_3$ group is axial. As the antibacterial activity of fortimicin A is much greater than that[3,4] of fortimicin B, our interest was directed towards the preparation of a series of 4-*N*-acylfortimicins B (**3**) in which the 4-*N*-glycyl group of fortimicin A is replaced by other acyl groups, in particular those derived from other amino acids.

RESULTS AND DISCUSSION

Preliminary studies indicated that direct selective 4-*N*-acylation of fortimicin B was not feasible. The preparation of 4-*N*-acylfortimicins thus required protection

TABLE I

N-BENZYLOXYCARBONYL PROTECTED 4-*N*-ACYLFORTIMICIN ANALOGS

Compound	*R'*	*Coupling method*	*Chromatography system*	*Isolated Yield (%)*	$[\alpha]_D^{23-25}$ *(c 1.0)*	*N.m.r. (δ) $CDCl_3$*	ν_{max} *(cm^{-1})*	*Empirical formula*	*Anal. Calc. C; H; N (Found C; H; N)*
6a	$-CH_2NHZ$	B	*A*	69	+52.9° (CH_3OH)	1.16 (6'-CH_3, *J* 6.5 Hz), 2.82 (4-NCH_3), 3.31 (3-OCH_3), 4.80 (H-1', *J* 3.0 Hz)	1710 1635 1500	$C_{49}H_{59}N_5O_{14}$	62.48; 6.31; 7.43 (62.52; 6.49; 7.23)
		C	*B*	95					
6d	$-CH_2CH_2NHZ$	B	*A*, Sephadex	71	+42.9° (CH_3OH)	1.17 (6'-CH_3, *J* 6.0 Hz), 2.82 (4-NCH_3), 3.28 (3-OCH_3), 4.78 (H-1')	1710 1620 1503	$C_{50}H_{61}N_5O_{14}$	62.82; 6.43; 7.32 (62.11; 6.47; 7.29)
6e	Me | CH_2N-Z	B	*A*, Sephadex	83	+49.9° (CH_3OH)	1.5 (6'-CH_3, *J* 6.8 Hz), 2.79 (4-NCH_3), 2.98 (3-OCH_3), 3.35 (2"-NCH_3), 4.82 (H-1', *J* 3.0 Hz)	1710 1635 1500	$C_{50}H_{61}N_5O_{14}$	62.82; 6.43; 7.32 (62.59; 6.47; 7.32)
6f	CH_2NMe_2	B	*C*	54	+46.1° (CH_3OH)	1.16 (6'-CH_3, *J* 6.0 Hz), 2.3 (2"-NMe_2), 2.89 (4-NCH_3), 3.06 (CO-CH_2-N<), 3.34 (3-OCH_3), 4.82 (H-1', *J* 3.0 Hz)	1711 1630 1503	$C_{43}H_{57}N_5O_{12}$	61.78; 6.87; 8.38 (61.75; 7.02; 8.30)
6g	NHZ (D) | -CHMe	B	*A, I*	24	+41.4° (CH_3OH)	1.15 (6'-CH_3, *J* 6.8 Hz), 1.28 (2"-CH_3, *J* 6.5 Hz), 2.88 (4-NCH_3), 3.27 (3-OCH_3), 4.82 (H-1', *J* 3.7 Hz)	1710 1625 1498	$C_{50}H_{61}N_5O_{14}$	62.82; 6.43; 7.32 (62.83; 6.59; 7.09)
6h	NHZ (L) | -CHMe	B	*A, D* Sephadex	47	+37.5° (CH_3OH)	1.17 (6'-CH_3, *J* 6.5 Hz), 1.27 (2"-CH_3, *J* 7.0 Hz), 2.97 (4-NCH_3), 3.29 (3-OCH_3), 4.77 (H-1', *J* 3.0 Hz)	1712 1630 1500	$C_{50}H_{61}N_5O_{14}$	62.82; 6.43; 7.32 (62.80; 6.58; 7.10)
6i	OH (DL) | $-CH-CH_2CH_2NHZ$	E	*E, I* Sephadex	28	+42.4° (CH_3OH)	1.19 (6'-CH_3), 2.90 (4-NCH_3), 3.32 (3-OCH_3), 4.75 (H-1', *J* 3.0 Hz)	1705 1623 1504	$C_{51}H_{63}N_5O_{15}$	62.12; 6.44; 7.10 (62.07; 6.54; 7.07)
6j	-Me	A	None	100	+58.4° (CH_3OH)	1.16 (6'-CH_3, *J* 6.0 Hz), 2.07 (Ac), 2.83 (4-NCH_3), 3.34 (3-OCH_3), 4.81 (H-1', *J* 3.0 Hz)	1710 1620 1500	$C_{41}H_{52}N_4O_{12}$	62.11; 6.61; 7.07 (62.37; 6.74; 7.00)

Table I (continued)

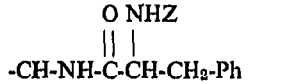
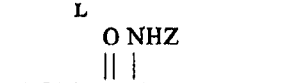
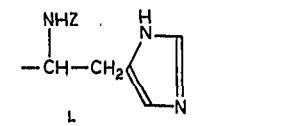
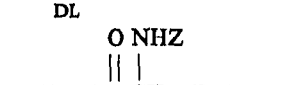
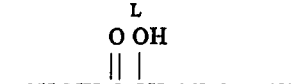
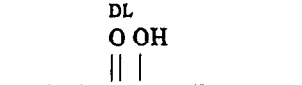
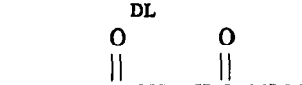
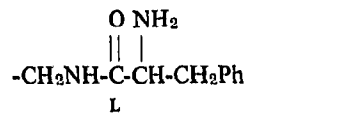
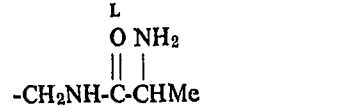
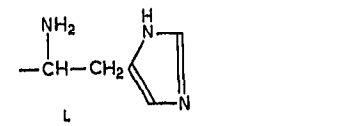
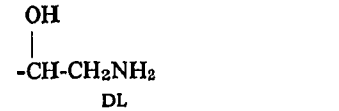
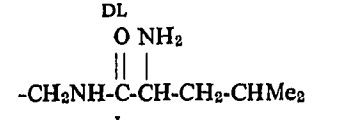
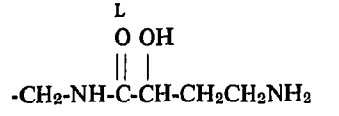
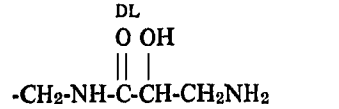
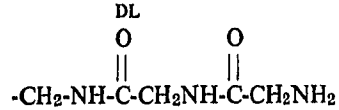
6k		B	*E*	88	+43° (CH_3OH)	1.17 (6′-CH_3, *J* 6.0 Hz), 2.87 (4-NCH_3), 3.32 (3-OCH_3)	1712 1638 1500	$C_{51}H_{62}N_6O_{15}$	61.31; 6.25; 8.41 (61.35; 6.40; 8.28)
6l		B	*A*, Sephadex	44	+28.4° (CH_3OH)	1.16 (6′-CH_3, *J* 6.0 Hz), 2.80 (4-NCH_3), 3.27 (3-OCH_3)	1712 1637 1500	$C_{58}H_{68}N_6O_{15}$	63.96; 6.29; 7.72 (63.82; 6.45; 7.71)
6m		B	*A*	79	+30.0° (CH_3OH)	~1.17 (6′-CH_3), ~1.29 (COCHNHZCH_3), 2.85 (4-NCH_3), 3.30 (3-OCH_3)	1711 1640 1500	$C_{52}H_{64}N_6O_{15}$	61.35; 6.37; 8.30 (61.68; 6.52; 8.28)
6n		F	*F*, *K*, Sephadex	40	+32° ($CHCl_3$)	1.15 (6′-CH_3), 2.91, 2.93 (4-NCH_3), 3.22, 3.29 (3-OCH_3), 5.03, 5.07 (Z-CH_2), 7.1–7.4 (Z aromatic)	1710 1631 1505	$C_{53}H_{63}N_7O_{14}$	62.28; 6.21; 9.59 (62.05; 6.31; 9.44)
6o		D	*G*, *H*, Sephadex	39	+43° ($CHCl_3$)	3.03 (4-NCH_3), 3.36, 3.31 (3-OCH_3), 5.0–5.1 (Z-CH_2), 7.2–7.4 (Z aromatic)	1705 1628 1500	$C_{50}H_{61}N_5O_{15}$	61.78; 6.33; 7.20 (61.71; 6.58; 7.27)
6p		D	*G*, Sephadex	91	+24° ($CHCl_3$)	0.92 (Leu-CH_3), 1.17 (6′-CH_3, *J* 6.0 Hz) 2.82 (4-NCH_3), 3.30 (3-OCH_3), 5.0–5.1 (Z-CH_2), 7.2–7.4 (Z aromatic)	(KBr) 1710 1636 1500	$C_{55}H_{70}N_6O_{15}$	62.60; 6.69; 7.96 (62.31; 6.78; 7.93)
6q		E	*G*, Sephadex	38	+29° ($CHCl_3$)	2.90, 2.99 (4-NCH_3), 3.32 (3-OCH_3), 5.0–5.1 (Z-CH_2), 7.2–7.4 (Z aromatic)	(KBr) 1710 1638 1510	$C_{53}H_{66}N_6O_{16}$	61.02; 6.38; 8.06 (60.80; 6.44; 8.02)
6r		E	*G*, Sephadex	80	+32° ($CHCl_3$)	2.90, 2.96 (4-NCH_3), 3.31 (3-OCH_3), 5.01–5.1 (Z-CH_2), 7.2–7.4 (Z aromatic)	1705 1636 1503	$C_{52}H_{64}N_6O_{16}$	60.68; 6.27; 8.17 (60.86; 7.47; 8.20)
6s		E	*G*, Sephadex	77	+44° ($CHCl_3$)	2.95 (4-NCH_3), 3.33 (3-OCH_3), 5.0–5.1 (Z-CH_2), 7.2–7.4 (Z aromatic)	1705 1670 1505	$C_{53}H_{65}N_7O_{16}$	60.27; 6.20; 9.28 (60.09; 6.22; 9.14)

TABLE II

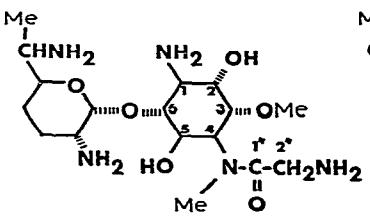
4-*N*-ACYLFORTIMICIN PERHYDROCHLORIC ACID SALT ANALOGS

Compound	*R*	$[\alpha]_D^{20-26}$ (*c 1.0*)	*N.m.r.* (*δ*) D_2O	ν_{max}^{KBr} (cm^{-1})	*Mass spectrum*
3a	$-CH_2NH_2$	+82.3° (CH_3OH)	1.79 (6′-CH_3, *J* 7.0 Hz), 3.57 (4-NCH_3), 3.93 (3-OCH_3, 5.76 (H-1′, *J* 3.2 Hz)	1643, 1590, 1490	M_f^+ Calc. for $C_{17}H_{35}N_5O_6$: 405.2587 Meas: 405.2617
3b	$-CH_2CH_2NH_2$	+61.3° (CH_3OH)	1.81 (6′-CH_3), *J* 6.9 Hz), 3.61 (4-NCH_3), 3.96 (3-OCH_3), 5.79 (H-1′, *J* 3.0 Hz)	1610, 1487	M_f^+ Calc. for $C_{18}H_{37}N_5O_6$: 419.2744 Meas: 419.2727
3c	$-CH_2NHMe$	+81.3° (CH_3OH)	1.84 (6′-CH_3, *J* 6.6 Hz), 3.32 (2″-NCH_3), 3.62 (4-NCH_3), 3.99 (3-OCH_3), 5.82 (H-1′, *J* 3.2 Hz)	1640, 1490	M_f^+ Calc. for $C_{18}H_{37}N_5O_6$: 419.2744 Meas: 419.2732
3d	$-CH_2NMe_2$	+79.3° (CH_3OH)	1.81 (6′-CH_3, *J* 6.4 Hz), 3.44, 3.47 [$N(CH_3)_2$], 3.56 (4-NCH_3), 3.95 (3-OCH_3), 5.80 (H-1′, *J* 3.0 Hz)	1640, 1600, 1484	M_f^+ Calc. for $C_{19}H_{39}N_5O_6$: 433.2900 Meas: 433.2903
3e	$-CH(NH_2)-Me$ D	+83.2° (CH_3OH)	1.81 (6′-CH_3, *J* 7.0 Hz), 2.01 (3″-CH_3, *J* 6.9 Hz), 3.69 (4-NCH_3), 3.94 (3-OCH_3), 5.77 (H-1′, *J* 3.7 Hz)	1632, 1600, 1475	M_f^+ Calc. for $C_{18}H_{37}N_5O_6$: 419.2744 Meas: 419.2723
3f	$-CH(NH_2)-Me$ L	+85.2° (CH_3OH)	1.81 (6′-CH_3, *J* 7.0 Hz), 1.97 (3″-CH_3, *J* 7.0 Hz), 3.69 (4-NCH_3), 3.95 (3-OCH_3), 5.80 (H-1′, *J* 3.8 Hz)	1640, 1600, 1495	M_f^+ Calc. for $C_{18}H_{37}N_5O_6$: 419.2744 Meas: 419.2723
3g	$-CH(OH)-CH_2CH_2NH_2$ DL		1.80 (6′-CH_3, *J* 6.5 Hz), 3.32, 3.64 (4-NCH_3), 3.94, 4.00 (3-OCH_3), 5.78, 5.93 (H-1′, *J* 3.8 Hz, *J* 3.6 Hz)	1600, 1487	M_f^+ Calc. for $C_{19}H_{37}N_5O_6$: 431.2744 Meas: 431.2762
3h	-Me	+87.2° (CH_3OH)	1.80 (6′-CH_3, *J* 6.9 Hz), 2.62 (Ac), 3.61 (4-NCH_3), 3.94 (3-OCH_3), 5.77 (H-1′, *J* 3.2 Hz)	1600	M+1 Calc. for $C_{17}H_{35}N_4O_6$: 391.2556 Meas: 391.2553
3i	$-CH_2NH-C(=O)-CH_2NH_2$	+70.5° (CH_3OH)	1.81 (6′-CH_3, *J* 6.4 Hz), 3.62 (4-NCH_3), 3.95 (3-OCH_3), 5.79 (H-1′, *J* 3.5 Hz)	1678, 1625, 1485	M_f^+ -H_2O Calc. for $C_{19}H_{36}N_6O_6$: 444.2676 Meas: 444.2699

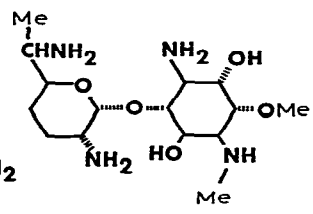
Table II (continued)

3j		+76.0° (CH_3OH)	1.80 (6′-CH_3, *J* 6.8 Hz), 3.59 (4-NCH_3), 3.94 (3-OCH_3), 5.77 (H-1′, *J* 3.5 Hz), 7.85 (Ph multiplet)	1674, 1627, 1490	M+1 Calc. for $C_{26}H_{45}N_6O_7$: 553.3350 Meas: 553.3329
3k		+76.9° (CH_3OH)	1.81 (6′-CH_3, *J* 6.5 Hz), 2.04 (-$CHNH_2CH_3$, *J* 7.2 Hz), 3.63 (4-NCH_3), 3.95 (3-OCH_3), 5.78 (H-1′, *J* 3.2 Hz)	1674, 1625, 1485	M^+ Calc. for $C_{20}H_{40}N_6O_7$: 476.2958 Meas: 476.2951
3l		+87° (CH_3OH)	1.81 (6′-CH_3, *J* 6.5 Hz), 3.61 (4-NCH_3), 3.92 (3-OCH_3), 5.79 (H-1′, *J* 3.5 Hz), 7.96 (His H-5, *J* 1.5 Hz), 9.22 (His H-2, *J* 1.5 Hz)	1640, 1590, 1490	M^+ -H_2O Calc. for $C_{21}H_{37}N_7O_5$: 467.2856 Meas: 467.2869
3m		+78° (CH_3OH)	1.83 (6′-CH_3, J 6.5 Hz), 3.75 (4-NCH_3), 3.99 (3-OCH_3), 5.82 (H-1′, *J* 3.5 Hz)	1625, 1487	M^+ -H_2O-NH_3 Calc. for $C_{18}H_{32}N_4O_6$: 400.2322 Meas: 400.2330
3n		+62° (CH_3OH)	1.45 (leu-CH_3, *J* 5.0 Hz), 1.81 (6′-CH_3, *J* 6.5 Hz), 3.63 (4-NCH_3), 3.96 (3-OCH_3), 5.79 (H-1′, *J* 3.5 Hz)	1670, 1630, 1487	M^+ Calc. for $C_{23}H_{46}N_6O_7$: 518.3428 Meas: 518.3454
3o		+58° (CH_3OH)	1.82 (6′-CH_3, *J* 6.5 Hz), 3.65 (4-NCH_3), 3.97 (3-OCH_3), 5.80 (H-1′, *J* 3.5 Hz)	1625, 1485	M^+ -3 H_2O Calc. for $C_{21}H_{36}N_6O_5$: 452.2747 Meas: 452.2767
3p		+68° (CH_3OH)	1.82 (6′-CH_3, *J* 6.5 Hz), 3.65 (4-NCH_3), 3.97 (3-OCH_3), 5.78 (H-1′, *J* 3.5 Hz)	1628, 1485	M^+ Calc. for $C_{20}H_{40}N_6O_8$: 492.2907 Meas: 492.2921
3q		+58° (CH_3OH)	1.74 (6′-CH_3, *J* 6.5 Hz), 3.56 (4-NCH_3), 3.89 (3-OCH_3), 5.81 (H-1′, *J* 3.5 Hz)	1635, 1485	M^+ -OH Calc. for $C_{21}H_{40}N_7O_7$: 502.2989 Meas: 502.2973

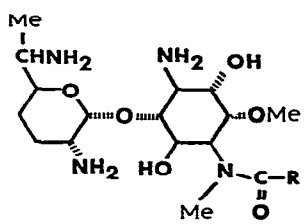
of the three primary amino groups with protecting substituents that could subsequently be removed under conditions which would not cleave the newly introduced, relatively labile, 4-*N*-acyl groups. For this purpose, we found the benzyloxycarbonyl group to be the protecting group of choice. The desired 1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B (**4**) was prepared directly from fortimicin B (**2**) by use of *N*-(benzyloxycarbonyloxy)succinimide (**5**). Alternatively, fortimicin A (**1**) was converted into 1,2′,6′,2″-tetra-*N*-benzyloxycarbonylfortimicin A (**6a**) with **5**, and the 4-*N*-(benzyloxycarbonyl)glycyl group of **6a** was selectively hydrolyzed to give 1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B (**4**). Acylation of the latter (**4**) at the methylamino group could be accomplished by standard methods. *N*-Benzyloxycarbonyl-protected aminoacyl groups were introduced by using such activated ester derivatives as *N*-hydroxysuccinimide[5,6] or *N*-hydroxy-5-norbornene-2,3-dicarboxamide esters[7], acyl azides[8], or 1-hydroxybenzotriazole esters[9,10], the latter being formed *in situ*.



1



2



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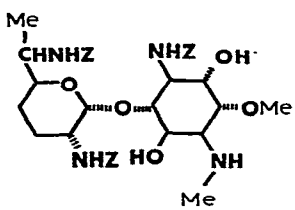

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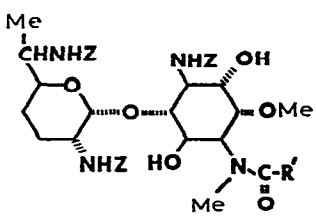

R′

6a CH_2NHZ

6b CH_2NH-t-BOC

6c CH_2NH_2

An alternative means of attaching peptide chains with glycine as the carboxyl terminus to the 4-methylamino group involved acylation of 1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B (**4**) with *N*-hydroxy-5-norborene-2,3-dicarboximide active ester of *N*-(*tert*-butyloxycarbonyl)glycine to form 4-*N*-(*N*-*tert*-butyloxycarbonylglycyl)-1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B (**6b**). Acid-catalyzed cleavage of the *tert*-butyloxycarbonyl group of the latter gave 1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin A (**6c**), isolated as the trifluoroacetate salt. Acylation of the glycyl nitrogen atom of **6c** was accomplished by standard methods.

The structures of the new, *N*-protected, fortimicin derivatives synthesized are shown in Table I. The compounds were conveniently isolated by column chromatography on silica gel and/or Sephadex LH-20. Each compound was characterized by microanalysis, n.m.r. and i.r. spectra, and optical rotation.

Selective removal of the benzyloxycarbonyl protecting groups of the resulting benzyloxycarbonyl-protected 4-*N*-acylfortimicins was accomplished by catalytic hydrogenation with palladium-on-carbon in 0.2M methanolic hydrochloric acid. Removal of the solvent gave the corresponding 4-*N*-acylfortimicins B (**3**), which were isolated as their perhydrochloric acid salts. The structures of the new fortimicin analogs prepared are shown in Table II. Each compound was fully characterized by n.m.r., i.r., and mass spectrometry.

TABLE III

ANTIMICROBIAL ACTIVITY OF THE PERHYDROCHLORIC ACID SALTS OF FORTIMICIN A AND FORTIMICIN ANALOGS

Compound	*Minimum inhibitory concentration*[a] *(μg/mL)*					
	Staphylococcus aureus *(Smith)*	Enterobacter aerogenes *13048*	Escherichia coli *(Juhl)*	Klebsiella pneumoniae *10031*	Shigella sonnei *9290*	Proteus mirabilis *Fin. No. 9*
3a	0.78	1.56	3.1	1.56	6.2	6.2
3b	3.1	6.2	6.2	6.2	6.2	12.5
3c	3.1	6.2	6.2	6.2	6.2	12.5
3d	12.5	>100	25	>100	50	>100
3e	12.5	100	100	>100	50	>100
3f	12.5	25	25	50	12.5	25
3g	100	>100	>100	>100	>100	>100
3h	>100	>100	>100	>100	>100	>100
3i	12.5	25	25	25	25	50
3j	>100	>100	100	>100	50	>100
3k	50	12.5	25	50	12.5	25
3l	50	>100	>100	>100	>100	>100
3m	25	50	50	50	50	50
3n	6.2	50	12.5	50	25	>100
3o	6.2	25	100	25	50	>100
3p	6.2	25	50	50	100	>100
3q	3.1	25	25	25	50	50

[a]The minimum inhibitory concentration of the fortimicins was determined by the dilution method with Mueller–Hinton agar, pH 7.4. The replicating device of Steers *et al.*[11] was used for inoculation.

To test the validity of the 4-*N*-acylation procedure, 1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B (**4**) was treated with both the *N*-hydroxysuccinimide and 1-hydroxybenzotriazole esters of *N*-benzyloxycarbonylglycine to give, in both cases, 1,2′,6′,2″-tetra-*N*-benzyloxycarbonylfortimicin A (**6a**) (Table I). The latter product was identical in all respects with material prepared directly from natural fortimicin A (**1**) as already described. Catalytic hydrogenation of synthetic **6a**, followed by conversion of the salt into the free base, gave fortimicin A (**1**), identical with natural material isolated from the fermentation.

Although fortimicin A and the 4-*N*-acylfortimicin B derivatives are relatively stable as their sulfate or hydrochloride salts, they are subject to decomposition as the free bases. The nature of the degradation of the free bases in aqueous solution will be described in a future report.

The 4-*N*-acylfortimicins B reported here were assayed as their perhydrochloride salts against a variety of bacteria (Table III). The most active derivatives, 4-*N*-sarcosyl- and 4-*N*-β-alanyl-fortimicin B (**3c** and **3b**, respectively), were approximately 50% as active as the parent fortimicin A (**1**). None of the 4-*N*-acylfortimicins exhibited a significantly broadened antibacterial spectrum.

EXPERIMENTAL

General methods. — All evaporations were conducted with a rotary evaporator under diminished pressure. Column chromatography on Sephadex LH-20 was effected with 95% aqueous ethanol. Silica gel column chromatography was performed on Silica Gel 60, 70–230 mesh (E. Merck Darmstadt) with the following solvent systems: *A* 23.5:1.4:2.0:0.2 (v/v) benzene–methanol–95% ethanol–concentrated ammonium hydroxide; *B*, 23.5:1.6:1.8:0.2 (v/v) benzene–methanol–95% ethanol–concentrated ammonium hydroxide; *C*, 18.2:1.8:0.2 (v/v) dichloromethane–95% aqueous methanol–concentrated ammonium hydroxide; *D*, 1:1 (v/v) acetone–hexane; *E*, 23.5:0.7:2.7:0.2 (v/v) benzene–methanol–95% ethanol–concentrated ammonium hydroxide; *F*, 117.0:7.0:0.5 (v/v) dichloromethane–95% aqueous methanol–concentrated ammonium hydroxide; *G*, 117.4:3.4:13.6:1.0 (v/v) benzene–methanol–95% ethanol–concentrated ammonium hydroxide; *H*, 85:15 (v/v) benzene–methanol; *I*, 1:1 (v/v) cyclohexane–acetone; *J*, 117.4:3.4:13.6 (v/v) benzene–methanol–95% ethanol; and *K*, 23.4:1.4:0.1 (v/v) chloroform–methanol–concentrated ammonium hydroxide.

Optical rotations were determined with a Hilger and Watts polarimeter. I.r. spectra were recorded with a Perkin–Elmer Model 521 grating spectrometer. N.m.r. spectra were determined at 100 MHz with a Varian Associates HA-100 spectrometer. Chemical shifts are reported in p.p.m. from internal tetramethylsilane ($\delta = 0.00$) and coupling constants are reported in Hz. Mass spectra were recorded with an A.E.I. MS-902 mass spectrometer with an ionization energy of 70 eV. Physical and analytical data are recorded in Table I for the protected derivatives, and in Table II for the deprotected products.

1,2′,6′,2″-Tetra-N-*benzyloxycarbonylfortimicin A* (**6a**). — A stirred, ice-bath cooled, solution of fortimicin A (**1**, 0.575 g), free base, freshly prepared from the disulfate salt, water (7.4 mL), and methanol (14.7 mL) was treated with 1.42 g of *N*-benzyloxycarbonyloxy)succinimide. Stirring was continued in the cold for 2.5 h and then for 20 h at room temperature. The major portion of the methanol was evaporated and the syrupy residue was shaken with a mixture of chloroform and water. The chloroform solution was separated, dried (magnesium sulfate), and evaporated. The residue was chromatographed on silica gel with System *A* to give 0.827 g of **6a**.

1,2′,6′-Tri-N-*benzyloxycarbonylfortimicin B* (**4**). — (*a*) *From fortimicin B* (**2**). To a stirred solution of 2.0 g of fortimicin B (**2**), water (30 mL) and methanol (60 mL), cooled in an ice-bath to 0°, was added *N*-(benzyloxycarbonyloxy)succinimide (4.44 g). Stirring was continued for 3 h at 0° and then for 22 h at room temperature. The major portion of the methanol was evaporated off and the syrup was shaken with a mixture of chloroform and water. The chloroform solution was washed with water, dried (magnesium sulfate), and evaporated to leave a residue that was chromatographed on a column of silica gel with System *K* to give 1.05 g of **4** as a white glass; $[\alpha]_D^{25}$ +16.5° (*c* 1.0, methanol); $\nu_{max}^{CDCl_3}$ 1712 and 1507 cm^{-1}; n.m.r. ($CDCl_3$): δ 1.03 (6′-CH_3, *J* 6.0 Hz), 2.32 (*N*HCH_3), and 3.41 (OCH_3).

Anal. Calc. for $C_{39}H_{50}N_4O_{11}$: C, 62.39; H, 6.71; N, 7.46. Found: C, 62.16; H, 6.76; N, 7.43.

(*b*) *From 1,2′,6′,2″-tetra*-N-*benzyloxycarbonylfortimicin A* (**6a**). A stirred mixture of 1,2′,6′,2″-tetra-*N*-benzyloxycarbonylfortimicin A (**6a**, 0.20 g), 5% aqueous sodium hydrogencarbonate (1.0 mL), and methanol (10 mL) was boiled for 4 h under reflux. The mixture was cooled and shaken with a combination of 5% aqueous sodium hydrogencarbonate (100 mL) and chloroform (50 mL). The chloroform solution was separated and the aqueous portion was extracted with chloroform. The chloroform solutions were combined and dried (magnesium sulfate). Evaporation of the chloroform left 0.165 g of **4**, identical with that prepared as already described in Part *a*.

Preparation of N-*benzyloxycarbonyl-protected 4*-N-*acylfortimicins*. — The methods employed for 4-*N*-acylation of 1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B are illustrated by the following examples:

Method A. With acetic anhydride. 4-N-*Acetyl-1,2′,6′-tri*-N-*benzyloxycarbonylfortimicin B* (**6j**). — To a stirred, ice-bath-cooled solution of 1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B (**4**, 3.22 g) in methanol (225 mL) was added acetic anhydride (16 mL) during 0.25 h. Stirring was continued for 2 h in the cold and then for 2 h at room temperature. The methanol was evaporated off and the residual acetic acid and acetic anhydride were removed by evaporation of benzene and methanol from the residue to leave 3.63 g of **6j**.

Method B. With in situ*-formed 1-hydroxybenzotriazole esters. 4*-N-*Sarcosyl-1,2′,6′,2″-tetra*-N-*benzyloxycarbonylfortimicin B* (**6b**). — To a stirred solution of 1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B (**4**, 2.26 g), *N*-benzyloxycarbonylsarcosine (0.855 g), and 0.982 g of 1-hydroxybenzotriazole monohydrate in oxolane (12.0 mL) was added *N,N′*-dicyclohexylcarbodiimide (0.808 g). Stirring was continued for 24 h

at room temperature. Insoluble *N,N'*-dicyclohexylurea was removed by filtration. Evaporation of the oxolane gave a yellow residue that was chromatographed on a column of silica gel with System *A*.

Fractions enriched in **6e** were combined and rechromatographed on a column of Sephadex LH-20 to give 2.29 g of **6e**.

Method C. With N-*hydroxysuccinimide esters. 1,2',6',2"-Tetra*-N-*benzyloxycarbonylfortimicin A* (**6a**). — To a stirred solution of 1,2',6'-tri-*N*-benzyloxycarbonylfortimicin B (**4**, 4.02 g) in oxolane (40 mL), cooled to 0° in an ice-bath, was added 1.80 g of the *N*-hydroxysuccinimide ester of *N*-benzyloxycarbonylglycine. Stirring was continued for 4 h at 0° and then for 23 h at room temperature. The resulting solution was shaken with a mixture of chloroform and 5% aqueous sodium hydrogencarbonate. The chloroform solution was separated and washed with water. Evaporation of the chloroform left 5.18 g of a white glass that was chromatographed on a column of silica gel in System *A* to give 4.58 g of **6a**.

Method D. With N-*hydroxy-5-norbornene-2,3-dicarboximide esters. 1,2',6',3"-*N-*Benzyloxycarbonyl-4*-N-(DL-*3-amino-2-hydroxypropanoyl)fortimicin B* (**6o**). — The *N*-hydroxy-5-norbornene-2,3-dicarboximide active ester of *N*-benzylcarbonyl-DL-2-hydroxy-3-aminopropanoic acid was prepared according to the general procedure of Fujino, *et al.*[7]. A solution prepared from *N*-benzyloxycarbonyl-DL-2-hydroxy-3-aminopropanoic acid (1.44 g), *N*-hydroxy-5-norbornene-2,3-dicarboximide (1.11 g), and *N,N'*-dicyclohexylcarbodiimide (1.28 g) in 10 mL of 1 : 1 (v/v) oxolane–1,4-dioxane was stirred for 3 h. The *N,N'*-dicyclohexylurea that formed was filtered off and the resulting solution of the active ester was added to a flask containing 2.25 g of 1,2',6'-tri-*N*-benzyloxycarbonylfortimicin (**4**), which was then stirred for 48 h at room temperature. A small amount of *N,N'*-dicyclohexylurea that formed was separated by filtration and the filtrate was evaporated to afford 5.46 g of residue. The latter product was chromatographed on columns of silica gel with Systems *G* and *H* to give 1.08 g of **6o**. An analytical sample of **6o** was prepared by chromatography on Sephadex LH-20.

Method E. With the trifluoroacetate salt of 1,2',6'-tri-N-*benzyloxycarbonylfortimicin A* (**6c**). *4*-N-(DL-*2-Hydroxy-4-aminobutanoyl)glycyl-tetra*-N-*benzyloxycarbonylfortimicin B* (**6q**). — To a stirring, ice-bath-cooled solution of *N*-benzyloxycarbonyl-DL-2-hydroxy-4-aminobutanoic acid (0.40 g) and *N*-hydroxy-5-norbornene-2,3-dicarboximide (0.32 g) in 30 mL of 1 : 1 (v/v) oxolane-1,4-dioxane was added *N,N'*-dicyclohexylcarbodiimide (0.36 g) in 10 mL of 1 : 1 (v/v) oxolane–1,4-dioxane. The solution was stirred for 0.5 h in the cold and then for 2 h at room temperature. The *N,N'*-dicyclohexylurea formed was separated by filtration and the filtrate containing the active ester was added to a flask containing 1.06 g of the trifluoroacetate salt of 1,2',6'-tri-*N*-benzyloxycarbonylfortimicin A (**6c**) prepared from 4-*N*-(*N*-*tert*-butyloxycarbonylglycyl)-1,2',6'-tri-*N*-benzyloxycarbonylfortimicin B (**6b**). After cooling the flask in a salt–ice bath, 0.56 mL of triethylamine was added and the resulting mixture was stirred overnight at room temperature. An additional 0.3 mL of triethylamine was added and stirring was continued for 0.5 h. The *N,N'*-dicyclo-

hexylurea that formed was separated by filtration. The filtrate was evaporated to give 2.37 g of residue that was chromatographed on a column of silica gel with System *G* to yield 0.35 g of **6q**. An analytical sample of **6q** was prepared by chromatography on Sephadex LH-20.

*Method F. Using acyl azides. Tetra-*N*-benzyloxycarbonyl-4-*N*-L-histidyl-fortimicin B* (**6n**). — A solution of 1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B (**4**, 1.5 g) in ethyl acetate (5 mL) was cooled in a Dry Ice–acetone bath and a cold solution of *N*-benzyloxycarbonyl-L-histidyl azide in ethyl acetate (19 mL), prepared from 1.21 g of *N*-benzyloxycarbonyl-L-histidylhydrazide by the procedure of Schneider *et al.*[8], was added with stirring. The mixture was stirred for 40 min at −15°, and then for 24 h at 4° and finally for 18 h at room temperature. Two drops of concentrated ammonium hydroxide was added, and the solvent was evaporated to leave 2.36 g of residue, which was chromatographed on a column of silica gel with System *F* to give 1.1 g of **6n**. An analytical sample of **6n** was prepared by chromatography on Sephadex LH-20.

Hydrogenolyses of N*-benzyloxycarbonylfortimicins.* — The general procedure employed for removal of the *N*-benzyloxycarbonyl groups is illustrated by the following example:

*4-*N*-Sarcosylfortimicin B tetrahydrochloride* (**3c**). — 4-*N*-Sarcosyl-1,2′,6′,2″-*N*-benzyloxycarbonylfortimicin B (**6e**, 0.840 g), 0.80 g of 5% palladium-on-carbon and 150 mL of 0.2M hydrochloric acid in methanol was hydrogenated for 4 h under 3 atm of hydrogen. The catalyst was removed by filtration and the methanol was evaporated. Residual water and hydrochloric acid were removed by repeated evaporation of methanol from the residue under diminished pressure, to give 0.529 g of **3c** as the perhydrochloric acid salt.

*1,2′,6′-Tri-*N*-benzyloxycarbonyl-4-*N*-(*N*-tert-butyloxycarbonylglycyl)fortimicin B* (**6b**). — A solution prepared from 1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B (**4**, 3.01 g) and 3.03 g of the *N*-hydroxy-5-norbornene-2,3-dicarboximide active ester of *N-tert*-butyloxycarbonylglycine in chloroform (10 mL) was stirred for 3 h in the cold and then for 18 h at room temperature. Evaporation of the solvent gave 6.84 g of residue, which was chromatographed on a column of silica gel with System *F* followed by chromatography on a column of Sephadex LH-20. Fractions containing pure **6b** were taken to dryness to give 1.07 g of solid: $[\alpha]_D^{26}$ +36° (*c* 1.05, chloroform); ν_{max}^{KBr} 1712, 1640, and 1500 cm^{-1}; n.m.r. ($CDCl_3$): δ 1.44 (*tert*-butyloxy-CH_3), 2.82 (4-NCH_3), 3.32 (3-OCH_3), 5.0–5.1 (Z–CH_2), and 7.2–7.4 (Z aromatic).

Anal. Calc. for $C_{46}H_{51}N_5O_{14}$: C, 60.84; H, 6.77; N, 7.71. Found: C, 60.52; H, 6.99; N, 7.66.

*1,2′,6′-Tri-*N*-benzyloxycarbonyl-4-*N*-glycylfortimicin B* (**6c**) *isolated as the trifluoroacetate salt.* — A solution prepared from 0.78 g of 4-*N-tert*-butoxycarbonylglycyl-1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B (**6b**), dichloromethane (5 mL), and 5 mL of trifluoroacetic acid was stirred for 20 min at room temperature. The solution was evaporated and dichloromethane was repeatedly evaporated from the residue. The residue was dried in high vacuum over potassium hydroxide and phos-

phorous pentaoxide for several h to give 1.06 g of the trifluoroacetate salt of **6c**, which was used without further purification.

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